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You can find your partners, iNtRON Distributor in Page.

High Quality
High quality plasmid DNA purified through our specially treated plasmid DNA-specific silica bead membrane. Minimal nicks of plasmid DNA guarantees good results in plasmid DNA sequencing.

Improved Recovery
Improved DNA extraction yields - from short length (3 Kb) to highly long length plasmid (34 Kb)

Prevention of error
Using a simple visual identification system, LysisViewer prevents common handling errors that lead to inefficient cell lysis and incomplete precipitation of SDS, cell debris, and genomic DNA.

Speed
Takes only 30 minutes to extract plasmid DNA.

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Sangdaewon-dong, Joongwon-gu,
Sungnam-si, Kyeonggi-do, Korea
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DNA Extraction | April, 2014 (7th Edition)

ISO 9001 | ISO 14001

Instruction manual

DNA-spin™ Plasmid DNA Purification Kit

The Instruction Manual for Plasmid DNA Extraction from *E. coli* using alkali lysis method and silica membrane.

REF	17096	Σ 50
REF	17097	Σ 200
REF	17098	Σ 200
RUO		
RT		

Product info.

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◈ Molecular Reagent / Molecular diagnosis

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DESCRIPTION

- DNA-spin™ Plasmid DNA Purification Kit provide a fast, efficient method of preparing high purity plasmid DNA without specialized devices or equipments.
- This kit contains a spin-type column filled with silica bead membrane and reagents optimizing alkali lysis for easy purification of plasmid DNA from bacteria. DNA-spin™ column has a specialized silica gel membrane that binds up to 35 μg (maximum) DNA in the presence of a high concentration of chaotropic salt and that allows elution in a small volume of low-salt buffer. The membrane technology eliminates time consuming phenol-chloroform extraction and alcohol precipitation, as well as the problems and inconvenience associated with loose resins and slurries.
- High-purity plasmid DNA eluted from DNA-spin™ columns is immediately ready to use - there is no need to precipitate, concentrate, or desalt.

CHARACTERISTICS

- **High Quality**

  High quality plasmid DNA purified through our specially treated plasmid DNA-specific silica bead membrane. Minimal nicks of plasmid DNA purified with our kit guarantees good results in plasmid DNA sequencing.

- **Improved Recovery**
  Improved the DNA extraction yields - from short length (3 Kb) to long length plasmid (34 Kb)

- **Prevention of error**
  Using a simple visual identification system, LysisViewer prevents common handling errors that lead to inefficient cell lysis and incomplete precipitation of SDS, cell debris, and genomic DNA.

- **Fast**
  Takes only 30 minutes to extract plasmid DNA.

KIT CONTENTS

Label	Contents 50 Columns	Contents 200 Columns
Resuspension Buffer [1]	15 ml	55 ml
Lysis Buffer [2]	15 ml	55 ml
Neutralization Buffer [3]	20 ml	80 ml
Washing Buffer A [4]	30 ml	140 ml
Washing Buffer B [5]	10 ml	40 ml
Elution Buffer [6]	20 ml	20 ml
DNA-spin™ column [7] (Yellow, w/o cap)	50 columns (17096)	200 columns (17097)
DNA-spin™ column [7] (With cap)		200 columns (17098)
Collection tube [8]	50 tubes	200 tubes
RNase A (Lyophilized powder) [9]	9 mg	33 mg
LysisViewer [10]	60 µl	220 µl

1 Before use, add reconstituted RNase A solution to Resuspension Buffer. Then, store at 4℃.

2 Check Lysis Buffer for SDS precipitation due to low storage temperature, in which case it is necessary to dissolve the SDS by warming to 37℃.

3 This buffer contains acetic acid and chaotropic salt.

4 *end*A+ strains such as HB101, the JM series strains, PR series strains and some other wide-type strains have high endonucleases activity. Endonucleases that can degrade plasmid DNA would be essentially removed by Washing Buffer A of DNA-spin™ Kit.

5 ***Before use, add 40ml (160ml) of absolute EtOH to the washing buffer B before use.***

6 DNase / RNase free Ultra-Pure solution.

7 The Columns containing silica membrane

8 Polypropylene tube for 2ml volume

9 The amount of lyophilized RNaseA provided is sufficient for the volume of Resuspension Buffer supplied with the kit. After receiving the kit, dissolve the lyophilized enzyme with Pure DW, then mix with Resuspension Buffer.

10 LysisViewer can be added to the Resuspension buffer bottle before use. Alternatively, smaller amounts of LysViewer can be added to aliquots of Resuspension buffer, enabling single plasmid preparations incorporating visual lysis control to be performed. LysisViewer should be added to Resuspension buffer at a ratio of 1:250 to achieve the required working concentration

◈ Suitable for Down-stream Operations

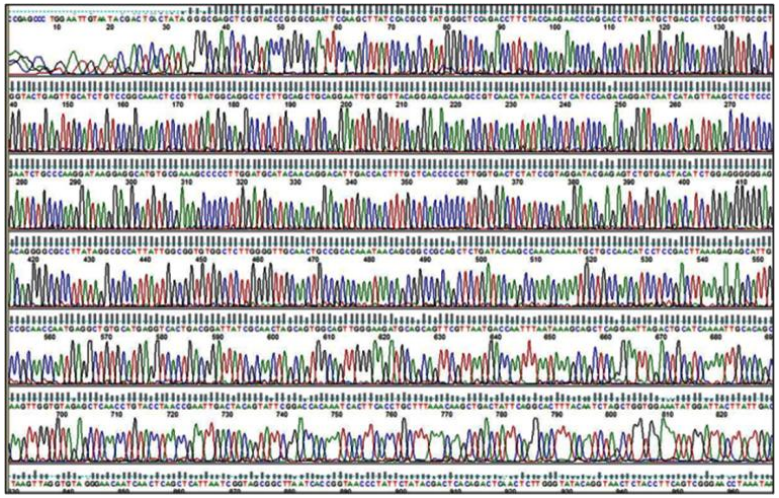


Fig. 4. Reliable Long Read Lengths in Sequencing
High quality sequencing data of pUC18 clones purified with iNtRON's DNA-spin™ Kit.

◈ Excellent Reproducibility

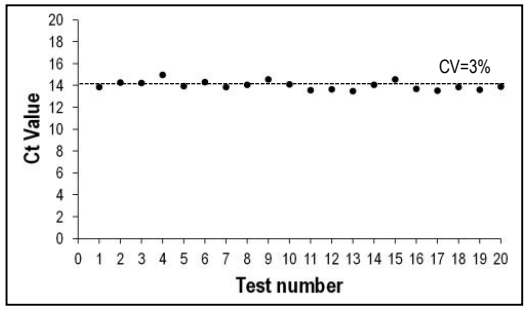


Fig. 5. The stability of DNA-spin™ Kit.
In order to estimate the stability of the DNA-spin™ Kit, tests were repeatedly performed 20 times, The recovery of 20 times repeated DNA extraction is very constant.

◈ Dependence of plasmid DNA yield from host strains

Table 1. Comparison of DNA Yields of plasmid DNA yield from host strains

Host strain	Recovery (μg)			
	iNtRON	Supplier A	Supplier B-1	Supplier B-2
DH5α	5.120	3.275	4.275	3.490
BL21	3.850	3.295	2.735	1.530
TOP10	4.935	3.340	3.090	3.365

Plasmid DNA extraction efficiencies in host strains were analyzed. pUC18 plasmid vector was introduced in each of the host strain, then applied with different commercial plasmid DNA Extraction Kits for estimating the purification efficiency. When using DNA-spin™ plasmid DNA Purification Kit, the DNA yields were determined approximately at 4 ~ 5 μg.

◈ Quality validation test of DNA-spin™ Kit with Competitor's products

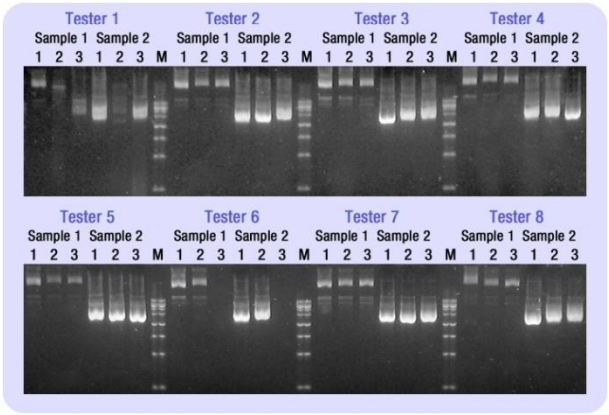

Tester 1
Sample 1 Sample 2
1 2 3 1 2 3 M
Tester 2
Sample 1 Sample 2
1 2 3 1 2 3 M
Tester 3
Sample 1 Sample 2
1 2 3 1 2 3 M
Tester 4
Sample 1 Sample 2
1 2 3 1 2 3
Tester 5
Sample 1 Sample 2
1 2 3 1 2 3 M
Tester 6
Sample 1 Sample 2
1 2 3 1 2 3 M
Tester 7
Sample 1 Sample 2
1 2 3 1 2 3 M
Tester 8
Sample 1 Sample 2
1 2 3 1 2 3

Fig. 3. Cross-check test for quality evaluation of DNA-spin™ Kit
In order to compare objective validity of the DNA-spin™ Kit, Eight of tester extracted 2 kinds of plasmid DNA (routine, long length) using DNA-spin™ Kit and Competitor's products. The DNA-spin™ Kit shows improved efficiencies for long length plasmid extraction.
Lane 1, DNA-spin™ Kit(iNtRON); **lane 2**, Supplier A; **lane 3**, Supplier B;
Sample 1, pADeasy (33.4 Kb); **Sample 2**, pUC18 (2.7 Kb)

STORAGE

DNA-spin™ Plasmid DNA Purification Kit should be stored at room temperature (15–25°C). Under these conditions, DNA-spin™ Plasmid DNA Purification Kit can be stored for up to 24 months without showing any reduction in performance and quality. The RNase A is shipped at room temperature and should be stored immediately upon receipt at 2–8°C. When stored at 2–8°C and handled correctly, the lyophilized enzyme can be stored for at least 24 months without any reduction in performance.

LYSISVIEWER

- Using a simple visual identification system, LysisViewer reagent prevents common handling errors that lead to inefficient cell lysis and incomplete precipitation of SDS, cell debris, and genomic DNA.
- LysisViewer can be added to the Resuspension Buffer bottle before use. Alternatively, smaller amounts of LysisViewer can be added to aliquots of Resuspension Buffer, enabling single plasmid preparations incorporating visual lysis control to be performed.
- LysisViewer reagent should be added to Resuspension Buffer at a ratio of 1:250 to achieve the required working concentration (e.g., 10 μl LysisViewer into 2.5 ml Resuspension Buffer). Make sufficient LysisViewer/Resuspension Buffer working solution for the number of plasmid preps being performed.
- LysisViewer precipitates after addition into Resuspension Buffer. This precipitate will completely dissolve after addition of Lysis Buffer. Shake Resuspension Buffer before use to resuspend LysisViewer particles.
- The plasmid preparation procedure is performed as usual. After addition of Lysis Buffer to Resuspension Buffer, the color of the mixed suspension changes to pink. Mixing should result in a homogeneously colored suspension. If the suspension contains localized regions of colorless solution or if brownish cell clumps are still visible, continue mixing the solution until a homogeneously colored suspension is achieved.
- Upon addition of Neutralization Buffer, LysisViewer turns colorless. The presence of a homogeneous solution with no traces of blue indicates that SDS from the lysis buffer has been effectively precipitated.

CONSIDERATION BEFORE USE

- **Lyophilized RNase A** : Dissolve the RNase A in 0.9 ml (3.3 ml for 200 Tests) of pure D.W. For long-term storage of reconstituted RNase A, remove the stock solution from the vial, divide it into single-use aliquots, and store at –20°C for up to 2 months. Thawed aliquots can be stored at 2–8°C for up to 12 weeks. Do not refreeze the aliquots after thawing
- Transformed bacteria should be inoculated in 3-5 ml of LB medium containing the appropriate antibiotics for selection and incubated in an appropriate conditions.

  Note : Incubate the culture at 37°C with vigorous shaking for 12-16 hrs. If you use ampicillin as an antibiotic for culture (OD_{600} 1.5-2.0), higher working ampicillin concentration to 200 ~ 300 μg/ml would be recommended to sustain selective antibiotic pressure for obtaining higher plasmid yield.
- Growth for more than 16 hr is not recommended since cells begin to lyse and plasmid yields may be reduced. Use a tube or flask with a volume of at least 4 times volume of the culture.
- If water is used for elution, make sure that its pH is between 7.0 and 8.5. Elution efficiency is dependent on pH and the maximum elution efficiency is achieved within this range. A solution with pH <7.0 can decrease yield.

  Note: Store DNA at –20°C when eluted with water, as DNA may degrade in the absence of a buffering agent.
- Buffers for Lysis, Neutralization and Washing A contain irritants. Wear gloves when handling these buffers.

SAFETY INFORMATION

All chemicals should be considered as potentially hazardous. When working with chemicals, always wear a suitable lab coat and disposable glove. Some buffer contain the chaotropic salt which may be an irritant and carcinogen, so appropriate safety apparel such as gloves and eye protection should be worn. If a spill of the buffers occurs, clean with a suitable laboratory detergent and water.
If the liquid spill contains potentially infectious agents, clean the affected area first with laboratory detergent and water, then with a suitable laboratory disinfectant. Only persons trained in laboratory techniques and familiar with the principles of good laboratory practice should handle these products.

DO NOT add bleach or acidic solutions directly to the sample preparation waste.

ADDITIONAL REQUIRED EQUIPMENT

- Absolute ethanol
- Standard tabletop microcentrifuge
- Microcentrifuge tubes, sterile (1.5 ml)

◈ Yields of High Copy and Low Copy Plasmid DNA

The iNtRON's DNA-spin™ Plasmid DNA Purification Kit were needed to reproduce in high yields with strains of *E. coli* harboring high-copy-number and low-copy-number plasmids carrying the pUC18 and pET40b, respectively.

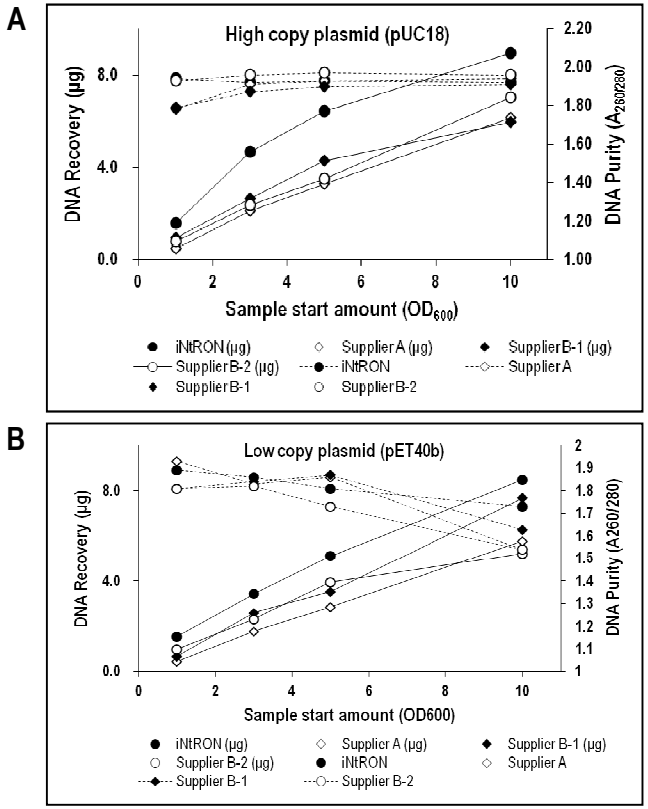
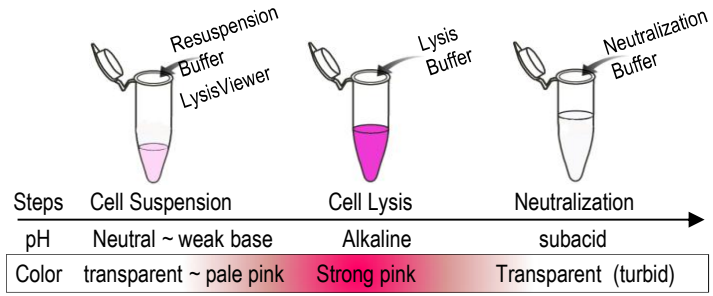


Fig 2. Comparison of DNA Yields of high copy and low copy plasmid DNA from Competitor's

E. coli pellets were harvested from bacterial cultures of 1 ml, 3 ml, 5 ml, and 10 ml (OD_{600} of 1.0). The yield of plasmid DNA extraction was proportional to increase culture volume. The optimal culture volume per one column is 3-5 ml at OD_{600} of 0.6-0.9.
A; High copy plasmid (pUC18), B; Low copy plasmid (pET40b).

EXPERIMENTAL INFORMATION

◈ Color Change of Alkaline Lysis Step with LysisViewer



◈ Yields of various sizes of plasmid DNA

DNA-spin™ Kit was used to purify plasmid DNA very efficiently from *E. coli* harboring various sizes of plasmid DNA from small (2.9 Kb) to large plasmid (33.4 Kb).

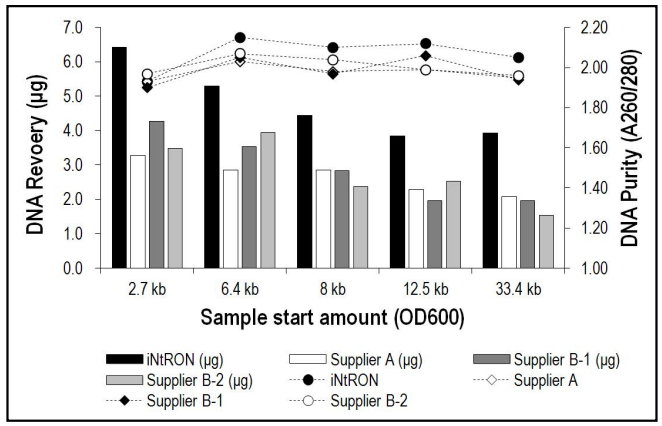


Fig. 1. Yield of plasmid DNA

Plasmid DNAs were extracted from 5 ml (OD_{600} of 1.0) of *E. coli* cultures containing 2.9 Kb, 6.5 Kb, 8 Kb, 12.5 Kb and 33.4 Kb plasmid DNA, respectively. And their purification yield and purity were analyzed. 1 μl of eluate were analyed on Nano-drop ND-2000 UV/VIS, spectrophotometically.

APPLICATIONS

Plasmid DNA prepared using the DNA-spin™ Plasmid DNA Purification Kit is suitable for a variety of routine applications including

- Restriction enzyme digestion
- Library screening
- In vitro translation
- Sequencing
- Ligation and transformation
- Transfection of robust cells

QUALITY CONTROL

- In accordance with iNtRON's ISO-certified Total Quality Management System, each lot of DNA-spin™ Plasmid DNA Purification Kit is tested against predetermined specifications to ensure consistent product quality.
- The quality of the isolated plasmid DNA was checked by restriction analysis, agarose gel electrophoresis, and spectrophotometric determination.
- DNA-spin™ column control
  The DNA binding capacity was tested by determining the recovery obtained with 20 μg of input high-copy–plasmid DNA. More than 70% recovery was obtained.
- RNase A
  Tested in plasmid purification. At concentrations up to 10 μg per mL, neither nicking nor degradation of plasmid are not detectable. One unit catalyzes the hydrolysis of RNA to yield an initial velocity constant of 1.0 at 25°C, pH 5.0
- Buffer control
  Conductivity and pH of buffers are as follows.

Buffer	Conductivity	pH
Resuspension	4.3 ~ 4.8 mS/cm	7.6 ~ 8.2
Lysis	39 ~ 45 mS/cm	10.9 ~ 11.5
Neutralization	154 ~ 170 mS/cm	2.8 ~ 3.1
Washing A	54 ~ 60 mS/cm	3.6 ~ 4.0
Washing B	11 ~ 13 mS/cm	7.4 ~ 7.8
Elution	550 ~ 620 μS/cm	8.0 ~ 8.5

SAMPLE PREPARATION

1. **Pick a single colony from a freshly streaked selective plate and inoculate a culture of 1–5 ml LB medium containing the appropriate selective antibiotic. Incubate for 12–16 hr at 37°C with vigorous shaking.**
   Note : If you use ampicillin as an antibiotic for culture (OD_{600} 1.5-2.0), higher working ampicillin concentration to 200 ~ 300 μg/ml would be recommended to sustain selective antibiotic pressure for obtaining higher plasmid yield.
   Note : Growth for more than 16 h is not recommended since cells begin to be lysed and plasmid yields may be reduced. Use a tube or flask with a volume of at least 4 times volume of the culture.
2. **Harvest the bacterial cells by centrifugation at > 8000 rpm in a table-top microcentrifuge for 3 min at room temperature (15–25°C).**
   Note : The bacterial cells can also be harvested in 15 ml centrifuge tubes at 5400 x g for 10 min at 4°C. Remove all traces of supernatant by inverting the open centrifuge tube until all medium has been drained.

COLUMN INFORMATION

• The DNA-spin™ Plasmid DNA Purification Kit Spin Column

Column membrane[1]	Silica-based membrane
Spin Column[1]	Individually, with a 2.0 ml Collection Tube
Max. Loading Volume	800 μl
Max. DNA Binding Capacity	45 μg
Recovery	85 - 95% depending on the elution volume
Elution Volume	Generally, eluted with 30 – 200 μl of elution buffer

⚠ 1. Do not store the Column packs under completely dried conditions. It may be affected to DNA binding capacity. The Spin Columns are stable for over 2 year under these conditions

TECHNICAL ASSISTANCE

At iNtRON we are proud of ourselves on the quality and availability of our technical support. Our Technical Service Departments are staffed by experienced scientists with extensive practical and theoretical expertise in molecular biology and the use of iNtRON products. If you have any questions or if you experience any difficulties regarding the G-spin™ Total DNA Extraction Kit or iNtRON products in general, please let us know.

cutting enzymes are used when compatible sticky-ended fragments cannot be generated – for example, if the polylinker site of a vector does not contain an enzyme site compatible with the fragment being cloned.

◈ Ligation of DNA

In order to construct new DNA molecules, DNA must first be digested using restriction endonucleases. The individual components of the desired DNA molecule are purified and then combined and treated with DNA ligase. The products of the ligation mixture are introduced into competent *E. coli* cells and transformants are identified by appropriate genetic selection. Appropriate control ligations should also be performed. Removal of 5' phosphates from linearized vector DNA can help prevent vector self-ligation and improve ligation efficiency. To remove 5' phosphates from DNA, add calf intestinal phosphate (CIP) buffer and 1 U CIP and incubate for 30–60 minutes at 37°C. Once the reaction is complete, inactivate CIP by heating to 75°C for 15 minutes.

1. **A typical ligation reaction is set up as follows:**
   - Component DNAs (0.1–5 μg)
   - Ligase buffer
   - 1 μl of 10 mM ATP
   - 20–500 U T4 DNA ligase

2. **Incubate for 1–24 hr at 15°C.**
   Note 1 : Simple ligations with two fragments having 4 bp 3' or 5' overhanging ends require much less ligase than more complex ligations or blunt-end ligations. The quality of the DNA will also affect the amount of ligase needed.
   Note 2 : Ligation of sticky-ends is usually carried out at 12–15°C to maintain a balance between annealing of the ends and the activity of the enzyme. Higher temperatures make annealing of the ends difficult, while lower temperatures diminish ligase activity.
   Note 3 : Blunt-end ligations are usually performed at room temperature since annealing is not a factor, though the enzyme is unstable above 30°C. Blunt-end ligations require about 10–100 times more enzyme than sticky-end ligations in order to achieve an equal efficiency.

3. **Introduce 1–10 μl of the ligated products into competent *E. coli* cells and select for transformants using the genetic marker present on the vector.**

4. **From individual *E. coli* transformants, purify plasmid DNAs by miniprep procedure and determine their structures by restriction mapping.**

◈ Storage of E. coli strains

There are different methods for storing *E. coli* strains depending on the desired storage time. Glycerol stocks and stab cultures enable long-term storage of bacteria, while agar plates can be used for short-term storage. When recovering a stored strain, it is advisable to check that the antibiotic markers have not been lost by streaking the strain onto an LB-agar plate containing the appropriate antibiotic(s).

◈ Restriction Endonuclease Digestion of DNA

DNA for downstream applications is usually digested with restriction endonucleases. This yields DNA fragments of a convenient size for downstream manipulations. Restriction endonucleases are bacterial enzymes that bind and cleave DNA at specific target sequences. Type II restriction enzymes are the most widely used in molecular biology applications. They bind DNA at a specific recognition site, consisting of a short palindromic sequence, and cleave within this site, e.g., AGCT (for *Alu*I), GAATTC (for *EcoR*I), and so on. Isoschizomers are different enzymes that share the same specificity, and in some cases, the same cleavage pattern.

Isoschizomers may have slightly different properties that can be very useful. For example, the enzymes *Mbo*I and *Sau*3A have the same sequence specificities, but *Mbo*I does not cleave methylated DNA, while *Sau*3A does. *Sau*3A can therefore be used instead of *Mbo*I where necessary.

Selecting suitable restriction endonucleases

The following factors need to be considered when choosing suitable restriction enzymes:

- Fragment size
- Blunt-ended/sticky-ended fragments
- Methylation sensitivity
- Compatibility of reaction conditions (where more than one enzyme is used)

Blunt-ended/sticky-ended fragments

Some restriction enzymes cut in the middle of their recognition site, creating blunt-ended DNA fragments. However, the majority of enzymes make cuts staggered on each strand, resulting in a few base pairs of single-stranded DNA at each end of the fragment, known as “sticky” ends. Some enzymes create 5' overhangs and others create 3' overhangs. The type of digestion affects the ease of downstream cloning:

- Sticky-ended fragments can be easily ligated to other sticky-ended fragments with compatible single-stranded overhangs, resulting in efficient cloning.
- Blunt-ended fragments usually ligate much less efficiently, making cloning more difficult. However, any blunt-ended fragment can be ligated to any other, so blunt-

PROTOCOL

- Add the dissolved RNase A solution to Resuspension Buffer, mix, and store at 2~8°C.
- Add ethanol (96–100%) to Washing Buffer B before use (see bottle label for volume).
- Check Lysis Buffer and Neutralization Buffer before use for salt precipitation. Redissolve any precipitate by warming to 37°C. Do not shake Lysis Buffer vigorously.
- Close the bottle containing Lysis Buffer immediately after use to avoid acidification of Lysis Buffer from CO_2 in the air.
- Lysis / Neutralization Buffer and Washing Buffer B contain irritants. Wear gloves when handling these buffers.
- Optional: Add the provided LysisViewer to Resuspension Buffer and mix before use. Use one vial of LysisViewer (spin down briefly before use) per bottle of Resuspension Buffer to achieve a 1:250 dilution. LysisViewer provides visual identification of optimum buffer mixing thereby preventing the common handling errors that lead to inefficient cell lysis and incomplete precipitation of SDS, genomic DNA, and cell debris.

1. **Pick a single colony from a freshly streaked bacterial plate and use it to inoculate LB (+antibiotics). And then grow at 37 ℃ for 12 ~ 16 hrs with vigorous shaking (OD_{600} = 1.0 ~ 1.5).**
   Note : Incubate the culture at 37°C with vigorous shaking for 12-16 hrs. If you use ampicillin as an antibiotic for culture (OD_{600} 1.5-2.0), higher working ampicillin concentration to 200 ~ 300 μg/ml would be recommended to sustain selective antibiotic pressure for obtaining higher plasmid yield.

2. **Harvest 3 – 5 ml of bacteria culture by centrifugation at 13,000 rpm for 30 sec at RT and discard supernatant.**
   Note : Drain tubes on a paper towel to remove excess media.

3. **Resuspend pelleted bacterial cell thoroughly in 250 μl of Resuspension Buffer by vortexing until no clumps remain.**
   Note : Ensure that RNase A solution has been added to Resuspension Buffer. It is essential to resuspend the cell pellet completely. It may affect the lysis efficiency.
   Note : If LysisViewer reagent is added to Resuspension Buffer, vigorously shake the buffer bottle to ensure LysisViewer particles are completely dissolved. The bacteria should be resuspended completely by vortexing or pipetting up and down until no cell clumps remain.

4. **Add 250 μl of Lysis Buffer to resuspended cells and mix by inverting the tube 10 times. DO NOT VORTEX and incubate for 3 min at RT.**
   Note : The optimal lysis time allows maximum release of plasmid DNA without release of chromosomal DNA, while minimizing the exposure of the plasmid to denaturing conditions. Long exposure to alkaline condition may cause the plasmid to become irreversibly denatured. It is important to proceed to next step immediately after the lysate becomes clear without any cloudy clumps. Do not vortex, it may cause shearing of genomic DNA.

Note : If the Lysis buffer becomes too cold, SDS precipitation may occur, leading to poor cell lysis. If a precipitate is observed, warm the Lysis buffer to 37°C with gentle shaking.
Note : If LysisViewer has been added to Resuspension Buffer, the cell suspension will turn pale pink after addition of Lysis Buffer. Mixing should result in a homogeneously colored suspension. If the suspension contains localized colorless regions or if brownish cell clumps are still visible, continue mixing the solution until a homogeneously colored suspension is achieved.

5. **Add 350 µl of Neutralization Buffer and gently mix by inverting the tube 10 times then incubate the tube in ice for 5 min.**
   Note : After addition of Neutralization Buffer, the solution should become cloudy and a fluffy white form. Incubation on ice may help precipitating the denatured cell components more efficiently. The precipitated material contains genomic DNA, protein, cell debris, and SDS.
   Note : If LysisViewer reagent has been used, the suspension should be mixed until all trace of pink has gone and the suspension is colorless. A homogeneous colorless suspension indicates that the SDS has been effectively precipitated.
6. **Centrifuge at 13,000 rpm for 10 min at 4℃. While waiting for the centrifugation, insert a column into collection tube.**
7. **After centrifugation, transfer supernatant promptly into the column.**
   Note : Cell debris, protein , and genomic DNA will form a compact white pellet in the tube.
8. **Centrifuge at 13,000 rpm for 1 min. Remove the column from collection tube, discard filtrate in collection tube. And then place the spin column back in the same collection tube.**
9. **(Optional) Add 500 µl of Washing Buffer A and centrifuge at 13,000rpm for 1 min. Remove the column from collection tube, discard filtrate in collection tube. And then place the spin column back in the same collection tube.**
   Note : This step is necessary to remove trace nuclease activity. endA+ strains, such as BL21, HB101, JM series, or any wild-type strains, have high level of nuclease activity that can degrade plasmids. But endA-strains,such as DH5α and XL1-blue, do not require this additional washing step.
10. **Add 700 µl of Washing Buffer B, centrifuge at 13,000 rpm for 1 min. Discard filtrate in the collection tube and place the spin column back in the same collection tube.**
11. **Centrifuge at 13,000 rpm for 1 min to dry the filter membrane.**
    Note : Remove ethanol completely. Residual ethanol from Washing Buffer B may inhibit subsequent enzymatic reaction.

◈ Host Strain

Most *E. coli* strains can be used successfully to isolate plasmid DNA, although the strain used to propagate a plasmid has an effect on the quality of the purified DNA. Host strains such as DH1, DH5α, and C600 give high-quality DNA. The slower growing strain XL1-Blue also yields DNA of very high-quality which works extremely well for sequencing. Strain HB101 and its derivatives, such as TG1 and the JM series, produce large amounts of carbohydrates, which are released during lysis and can inhibit enzyme activities if not completely removed. In addition, these strains have high levels of endonuclease activity which can reduce DNA quality. The methylation and growth characteristics of the strain should also be taken into account when selecting a host strain. XL1-Blue and DH5α are highly recommended for reproducible and reliable results.

◈ Antibiotics

Bacterial strains carrying plasmids or genes with antibiotic selection markers should always be cultured in liquid or on solid medium containing the appropriate selective agent. Lack of antibiotic selection can lead to loss of the plasmid carrying the genetic marker and potentially to selection of faster-growing mutants.

Antibiotic	Stock solutions		Working concentration (dilution)
	Concentration	Storage	
Ampicillin (sodium salt)	50 mg/ml in water	–20°C	100 µg/ml (1/500)
Chloramphenicol	34 mg/ml in ethanol	–20°C	170 µg/ml (1/200)
Kanamycin	10 mg/ml in water	–20°C	50 µg/ml (1/200)
Streptomycin	10 mg/ml in water	–20°C	50 µg/ml (1/200)
Tetracycline HCl	5 mg/ml in ethanol	–20°C	50 µg/ml (1/100)
Carbenicillin	50 mg/ml in water	–20°C	50 µg/ml (1/1000)

TECHNICAL ADVISE

◈ Growth of Bacterial Cultures

Plasmids are generally prepared from bacterial cultures grown in the presence of a selective agent such as an antibiotic. The yield and quality of plasmid DNA may depend on factors such as plasmid copy number, host strain, inoculation, antibiotic, and type of culture medium.

Note : Incubate the culture at 37°C with vigorous shaking for 12-16 hrs. If you use ampicillin as an antibiotic for culture (OD_{600} 1.5-2.0), we recommend to increase of your working ampicillin concentration to 200 ~ 300 μg/ml to sustain selective antibiotic pressure for obtaining higher plasmid yield.

◈ Plasmid Copy Numbers

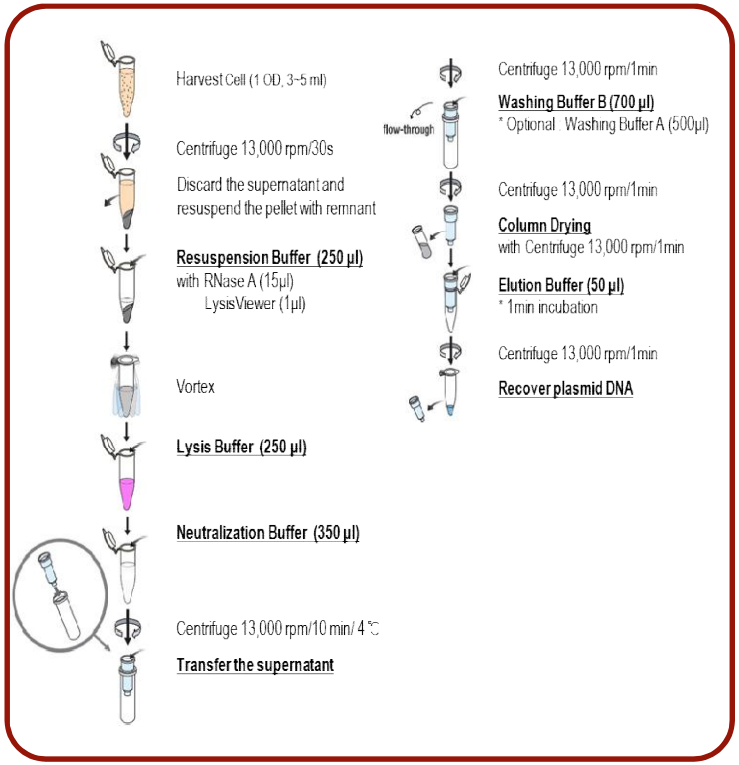
Plasmids show wide variation in their copy number per cell, depending on their origin of replication (e.g., pMB1, ColE1, or pSC101) which determines whether they are under relaxed or stringent control; and depending on the size of the plasmid and its associated insert. Some plasmids, such as the pUC series and derivatives, have mutations which allow them to reach very high copy numbers within the bacterial cell. Plasmids based on pBR322 and cosmids are generally present in lower copy numbers. Very large plasmids and cosmids are often maintained at very low copy numbers per cell.

DNA construct	Origin replication	Copy number	Classification
Plasmids			
pUC vectors	pMB1	500~700	high copy
pBluescript vectors	ColE1	300~500	high copy
pGEM® vectors	pMB1	300~400	high copy
pTZ vectors	pMB1	>1000	high copy
pBR322 and derivatives	pMB1	15~20	low copy
pACYC and derivatives	p15A	10~12	low copy
pSC101 and derivatives	pSC10	1 -5	very low copy
Cosmids			
SuperCos	ColE1	10–20	low copy
pWE15	ColE1	10–20	low copy

12. Put the column into a clean and sterile centrifuge tube. Add 50 μl of Elution Buffer or distilled water to the upper reservoir of the column, and let it stand for 1min. Then, centrifuge the tube assembly at 13,000 rpm for 1 min.

Note : It is suggested to use at least 30 μl of the Elution buffer to obtain best result. If the plasmid is low-copy -number or larger than 10 Kb, the yield of plasmid may not be sufficient. In this case, pre-warmed (about 50℃) elution buffer will improve efficiency of elution.

Quick Guide



TROUBLESHOOTING GUIDE

Problem	Possible Cause	Recommendation
Low or no yield	Plasmid did not propagate	• Check that the conditions for optimum growth were met
	Lysis buffer is precipitated	• Check the Lysis buffer for SDS precipitation due to low storage temperature and dissolve the SDS by warming to 37°C.
	Lysis buffer incompletely mixed	• Ensure complete mixing through all steps. When put and mix Lysis buffer and Neutralization buffer, do not shake vigorously
	Cell resuspension Incomplete	• Pelleted cells should be completely resuspended in Resuspension buffer. Do not add Lysis buffer until an even suspension is obtained.
	Step were not followed correctly or wrong reagent used	• Check the protocol; Washing buffer does not contain 100% EtOH, so, 100% EtOH must be added to the Washing buffer before use.
	Column was overloaded with DNA	• Check the culture volume and yield for use, and reduce the culture volume accordingly.
	Plasmid did not propagate	• Check the bacterial culture conditions (age of culture, antibiotics, culture volume and bioreactor)
RNA in the eluate	RNase A digestion omitted	• Ensure that RNase A is added to Resuspension Buffer before use
	RNase A digestion insufficient	• Reduce culture volume if necessary. If Resuspension Buffer containing RNase A is more than 6 months old, add additional RNase A

TROUBLESHOOTING GUIDE

Problem	Possible Cause	Recommendation
RNA in the eluate	RNase A digestion insufficient	• Check the KIT CONTENT and STORAGE; Resuspension buffer should be stored at 4℃ after adding RNase A solution. • Increase the incubation time after mixing with Neutralization Buffer for 3~5min
DNA is nicked /sheared/degraded	Endonuclease-containing host	• When put and mix Lysis buffer and Neutralization buffer, do not shake vigorously.
Problems in down-stream experiments	Salt sensitive application	• Such as blunt-end ligation and direct sequencing, Repeat the step 10 using 500 μl of Washing Buffer B.
Genomic DNA In the eluate	Lysis time was too long	• Ensure that the lysis step does not exceed 5min.
	Lysis Buffer added incorrectly	• The Lysate must be handled gently after addition of Lysis Buffer to prevent shearing. Reduce culture volume if lysate is too viscous for gentle mixing
	Neutralization Buffer added incorrectly	• Upon addition of Neutralization Buffer, mix immediately but gently
	Culture overgrown	• Overgrown cultures contain lysed cells and degraded DNA. Do not grow cultures for longer than 12~16 hours